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US 7541179		SNS23 Vectors
1.0	A recombinant vector comprising	The following vectors are each a "recombinant vector": SNS23.B87.A1 and SNS23.2.B87.A1 (collectively, the "SNS23 Vectors"). Exemplary support: US 7541179 ("the '179 Patent") defines "recombinant lentiviral vector" as follows: "The term 'recombinant lentiviral vector' refers to an artificially created polynucleotide vector assembled from a lentiviral-vector and a plurality of additional segments as a result of human intervention and manipulation." '179 Patent, at Col. 2: 36-40. US Patent Publication No. 2020/0291433 A1 ("the '433 Publication") identifies the SNS23 Vectors as recombinant vectors. For example, the '433 Publication states: "The presently disclosed subject matter provides vectors ... comprising the above-described expression cassettes. *The vectors ... are suitable delivery vehicles for the stable introduction of globin gene (e.g., human β-globin) into the genome of a broad range of target cells to increase expression of the globin protein (human β-globin protein) in the cell*." '433 Publ., at [0253] (italics added). "In certain embodiments, *the vector is a retroviral vector (e.g., gamma retroviral vector or a lentiviral vector) that is employed for the introduction or transduction of the above-described expression cassette into the genome of a host cell (e.g., a hematopoietic stem cell*, an embryonic stem cell, an induced pluripotent stem cell, or a hemogenic endothelium cell) . . ." *Id.*, at [0254] (italics added). The '433 Publication, Fig. 13, shows exemplary recombinant vectors, including SNS23.2.B87.A1. *Id.,* at [0245]. Fig. 15 also shows exemplary recombinant vectors, including SNS23.B87.A1. *Id.*, at [0246].
1.1	a nucleic acid encoding a functional globin	The following recombinant vectors each comprise "a nucleic acid encoding a functional globin": SNS23.B87.A1 and SNS23.2.B87.A1.

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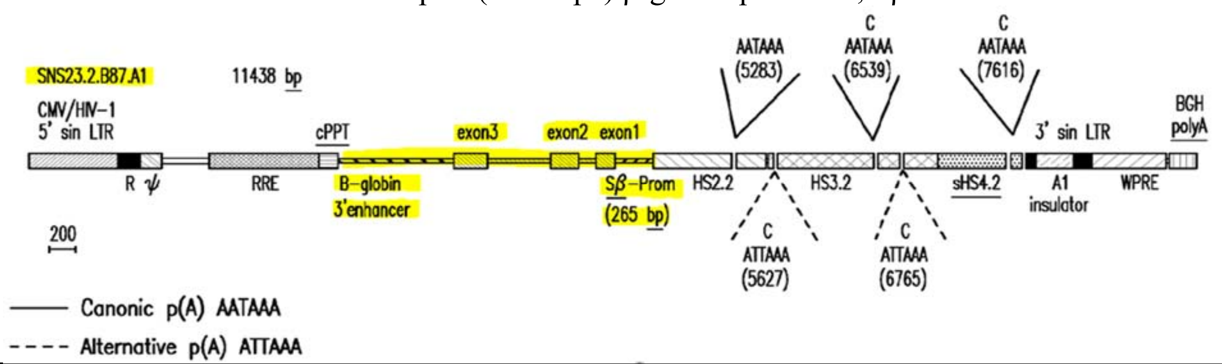
		<u>Exemplary support</u>: "The term 'functional globin gene' refers to a nucleotide sequence the expression of which leads to a globin that does not produce a hemoglobinopathy phenotype, and which is effective to provide therapeutic benefits to an individual with a defective globin gene." '179 Patent, at col. 2:41-44. "The functional globin gene may encode a wild-type globin appropriate for a mammalian individual to be treated, or it may be a mutant form of globin, preferably one which provides for superior properties, for example superior oxygen transport properties. *The functional globin gene includes both exons and introns, as well as globin promoters and splice donors/acceptors*. Suitably, the globin gene may encode α-globin, β-globin, or γ-globin. β-globin promoters may be sued [sic] with each of the globin genes." *Id.*, at col. 2:44-53 (italics added). The SNS23.B87.A1 and SNS23.2.B87.A1 vectors each encode a functional globin, as each vector contains within it β-globin exons 1 to 3, β-globin introns 1 and 2, plus a β-globin promoter and enhancer. Two figures from the '433 Publication (Figures 14 and 15) demonstrate these features and have been partially reproduced below, with their globin-related features highlighted in yellow. The excerpt below from Figure 14 demonstrates that the SNS23.2.B87.A1 vector has β-globin exons 1, 2 and 3, β-globin introns 1 and 2, as well as a β-globin 3' enhancer. SNS23.2.B87.A1 is also shown with the 265 base-pair ("265 bp") β-globin promoter, Sβ-Prom. 

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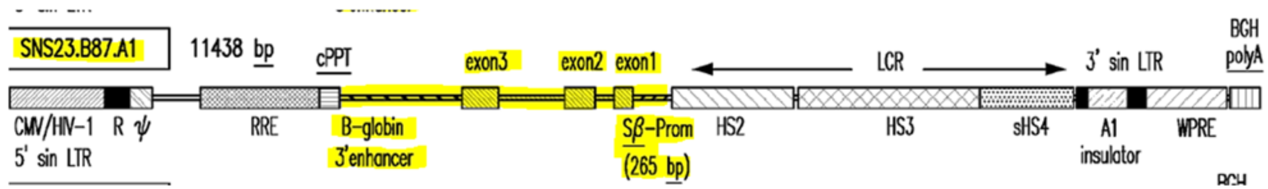
<table>
<tr><th colspan="2">US 7541179</th><th>SNS23 Vectors</th></tr>
<tr><td></td><td></td><td>
The excerpt below from Figure 15 demonstrates that the SNS23.B87.A1 vector has β-globin exons 1, 2 and 3, β-globin introns 1 and 2, the β-globin 3’ enhancer, and the 265 base-pair β-globin promoter, Sβ-Prom.



The SNS23.B87.A1 and SNS23.2.B87.A1 vectors (*e.g.*, Figs. 14-15) possess well-known control regions for human β-globin such as exons 1, 2, and 3, introns 1 and 2, and a β-globin enhancer and promoter, as described in the ’179 Patent, at col. 2: 41-51; accordingly, a POSA would understand that each of these vectors comprise “a nucleic acid encoding a functional globin.”

The ’433 Publication also provides additional information regarding the functionality and therapeutic usefulness of the β-globin produced by the SNS23.2.B87.A1 vector. *See, e.g.*, Figures 16 and 20, as shown on the following pages. The information specific to the SNS23.2.B87.A1 vector in these two figures has been highlighted in yellow.
</td></tr>
</table>

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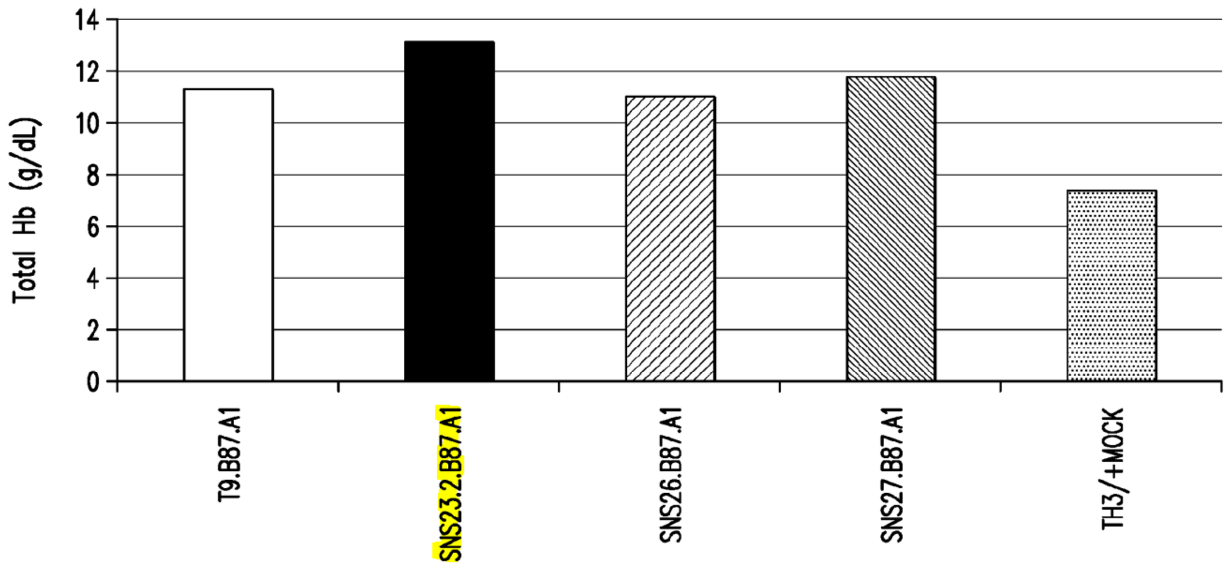
US 7541179		SNS23 Vectors
		"FIG. 16 depicts the average Hb production for vectors (TNS9.B87.A1, *SNS23.2.B87.A1*. . . ." '433 Publ., at [0059] (italics added).  *Average of total Hb: (TNS9.B87.A1 n=4, SNS23.2.B87.A1 n=5 SNS26.B87.A1=5 SNS27.2.B87.A1=5 $TH^{3}/^{+}$mock n=1)* FIG. 16

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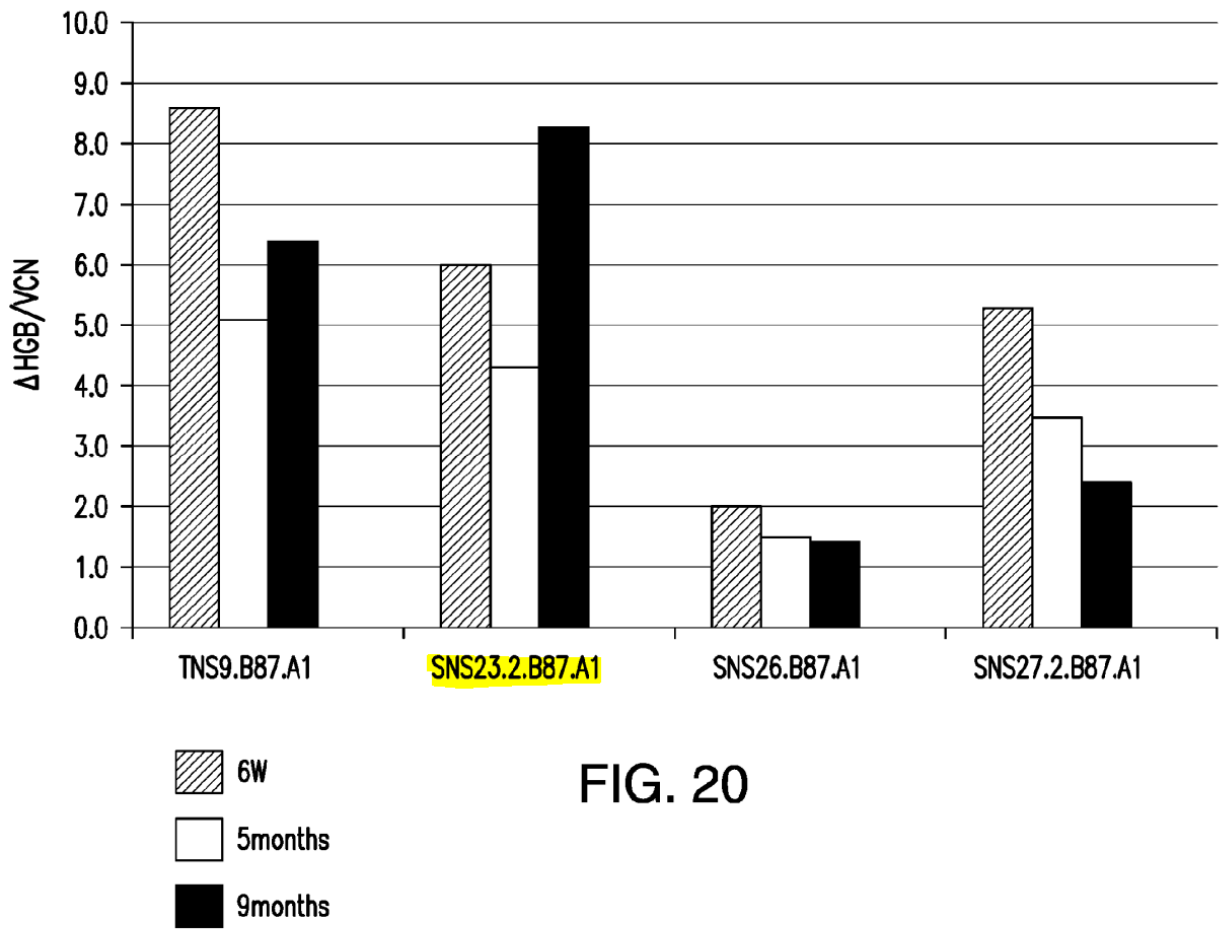
		"FIG. 20 depicts the average gain in Hb level (ΔHb) normalized to vector copy (VCN) at time points of 6-week, 5-month, and 9-month. ΔHb=Hb level−7.5 (baseline level in thalassemic mice)." '433 Publ., at [0063].  FIG. 20

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1.2	operably linked to a 3.2-kb nucleotide fragment which consists essentially of three contiguous nucleotide fragments obtainable from a human β-globin locus control region (LCR),	The SNS23.B87.A1 and SNS23.2.B87.A1 recombinant vectors each meet the claim limitation of being "operably linked to a 3.2-kb nucleotide fragment which consists essentially of three contiguous nucleotide fragments obtainable from a human β-globin locus control region (LCR)". Exemplary support: Each of these vectors performs the same function (enhancing β-globin expression beyond levels previously achieved), in the same way (to obtain the same result): through the incorporation of fragments of the HS2, HS3 and HS4 DNase I hypersensitive sites obtained from a human β-globin control region, which fragments are larger than the previously tested minimal HS core elements but smaller than about 3.2 kb when combined. A Person of Ordinary Skill in the Art ("POSA") would read the information in May et al., *Therapeutic haemoglobin synthesis in β-thalassemic mice expressing lentivirus-encoded human β-globin*, Nature, Vol 406, pp. 82-86 (July 6, 2000) and the prosecution history for US Patent Application No. 10/188221, and understand them as indicating that the new vectors described therein (and claimed in the '179 Patent) occupied a middle ground between the prior art vectors that contained minimal LCRs and the prior art vectors that contained much larger LCRs. For example, May 2000 states: "Incorporation of small elements spanning DNase HS2, HS3 and HS4 into viral vectors increases β-globin expression in mouse erythroleukaemia (MEL) cells[9,10]**.** *However, low-level expression, strong position effects and transcriptional inactivation are still observed in bone marrow chimaeras*[5,11]. Studies in transgenic mice[12] and deletional analyses[13] support the view that coordinated interaction of several genetic elements including the LCR is required for physiologic β-globin gene expression[12-15]. *We therefore thought that incorporation of large elements spanning HS2, HS3 and HS4*[16-18] *in a vector might enhance β-globin expression beyond levels previously achieved using arrayed minimal core elements*[5,9-11]*, and thus might diminish position effects and vector silencing. The efficient transduction of large genomic fragments using onco-retroviral vectors has proved to be severely curtailed by splicing and other alterations affecting the stability of the recombinant genomes*[9,10,16]. Here we report how these problems may be overcome by using vectors derived from human immunodeficiency virus 1, a retrovirus that has the ability to regulate packaging of unspliced viral genomes. We constructed two recombinant

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		lentiviruses carrying β-globin transcription units (Fig. 1a, b). RNS1 contains a minimal LCR comprising previously tested core elements of HS2, HS3 and HS4 (ref. 9).” May 2000, at p. 82 (italics added). This contention is supported by multiple statements in the prosecution history of the patent application that ultimately issued as the ’179 Patent (USSN 10/188221, hereafter “the ’221 Application”). For example, the Applicants stated: “The nature of the invention identifies the field of the endeavor - here a recombinant vector for treating hemoglobinopathies by expressing a functional globin in vivo using the claimed 3.2-kb portion of a human B-globin LCR. As an aside, ... the Examiner characterized the three fragments in the LCR as ‘essential elements from the B-globin LCR.’ Applicants wish to clarify this remark as it is not a term of art and is somewhat misleading. *The literature describes core HS sites as small fragments, and these core sequences might be considered as ‘essential’ or ‘minimal’ since they are the smallest fragments that can effect globin expression.* ***In point of fact, the present invention resides in having more than these small core sequences, namely, the invention resides in having the larger, specific HS-containing fragments in the vector and obtaining a level of globin expression not previously possible in vivo***.” 09/12/2007 Rule 116 Amendment and Response for US Patent Application No. 10/188221, at p. 13 (hereafter “09/12/2007 Response”) (italics and emphasis added). In fact, Applicants specifically pointed out to the USPTO Examiner that prior art vectors had human β-globin LCR fragments that ranged in size from very large (20 kb) to very small (1 kb): “The human β-globin Locus Control Region was known to be a 20-30 kb region extending upstream from the start of the ε-globin gene, and the scientific literature had reported “a variety of expression studies with a 20-kb ‘minilocus,’ a 6.5-kb [‘]microlocus’ and a 1-kb fragment with core DNAse I hypersensitive site.” 09/12/2007 Response, at p. 10 (internal citations omitted). The Applicants distinguished their claims from the prior art by contending that, to their knowledge, “no previous studies have been conducted with a 3.2-kb portion of a human β-globin LCR as claimed herein.” *Id*.

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		Because the descriptions of the core sequences for the HS2, HS3 and HS4 regions were known in the prior art, the Applicants asserted that not only should one assume the DNAse hypersensitivity-spanning fragments "are *at least as big as their corresponding core sequences", but "[i]n fact, one knows they must be larger*." 09/12/2007 Declaration of Jason W. Plotkin Under 37 C.F.R. §1.132 (hereafter, "Plotkin Declaration") at ¶ 36 (italics added), as submitted with 09/12/2007 Response. A POSA would know that incorporation of only the "core" LCR fragments resulted in vectors with low viral titers that were "highly unstable with multiple rearrangements of the transferred proviral structures". *See* Negre *et al*., 2016, *Gene Therapy of the β-Hemoglobinopathies by Lentiviral Transfer of the βA(T87Q)-Globin Gene*, Human Gene Therapy, Vol. 27, No. 2: 148- 165, at p. 154 (hereafter, "Negre 2015"). In addition, a POSA would have been aware that "[r]educing the size of the LCR to minimal elements is unsatisfactory as β-globin expression levels are too low." *Id*. (citing references from 1992-1997); *see also*, May 2000, at p. 82. Accordingly, a POSA would understand that the claimed vectors needed to have an HS2-HS3-HS4 LCR region that was bigger than 1 kb (*i.e*., had more than the minimal core HS sequences). In addition to setting a lower 1 kb boundary, the Applicants provided a flexible upper boundary of approximately 3.2 kb for the combined HS-spanning nucleotide fragments when they argued to the USPTO that: "The simple fact that the combination of the three HS-spanning fragments is 3.2 kb partially (and significantly) closes this aspect of the present claim, ***qualifies its size and thus provides the boundaries for ascertaining the elements excluded by use of "consisting essentially of" as the transitional phrase***. For example, any additional nucleotides added to the 3.2 kb fragment that cause the fragment to exceed 3.2 kb, would alter a basic and novel property of the invention. *As Applicants have exhaustively established on the record, the combined size of the three HS-spanning fragments so closely approximates 3.2 kb, that the number of additional nucleotides that could be added to (or removed from) this fragment is relatively few and non-material.* ***For example, the types of non-material nucleotide changes that can be accomodated are*** . . . ***adding a small linker to provide or change a restriction site, or making any other minor change to the sequence that does not alter the functionality of the fragment in driving globin expression, including changes at the ends of or at the junction points of the fragments***. All such changes are well known in the art and would be readily contemplated, accomplished and analyzed by

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		skilled artisans. ***However, none of these non-material changes rises to the level of the fragments taught by Ryan or Antoniu. . . . Ryan shows only a 30-kb and a 22-kb recombinant nucleotide fragement that contain at least HS2, HS3, and HS4. . . . . . . Antoniu show[s] only a single 5.5 kb nucleotide fragment that contains HS2, HS3 and HS4. Based on size, the Ryan and Antoniu fragments clearly differ from the 3.2 kb fragment of Claim 1***. Further, based on nucleotide composition and arragement of the HS fragments (i.e., which pieces of the LCR are present), *neither Ryan nor Antoniu shows any fragment that combines the recited HS2-, HS3- and HS4-spanning fragments in contiguity into a single 3.2-kb fragment as claimed in present Claim 1*. Ryan's fragments are single, large restriction fragments from the LCR encomapassing all 5 HS sites in their natural order and sequence context. Antoniu fragments combine various restriction fragments which are larger and and distinct from those claimed by Applicants. Merely because the three HS fragments that Applicants have identified are within the sequence of the Ryan and Antonius fragments does not mean that those references "encompass" the claimed 3.2-kb fragment and thereby anticipate the present invention. ***The actual combination must be demonstrated ... and it is not, as evidenced by Applicants' use of "consisting essentially of" as the transitional phrase, along with bounding this operable LCR fragment at 3.2 kb, which, therefore, serve to distinguish the claimed invention from Ryan and Antonius as well as establish the basic and novel properties of this nucleotide fragment***." 12/03/2008 Amendment and Response After Final Office Action for US Patent Application No. 10/188221, at pp. 9-10 (italics and emphasis added). Thus, a POSA would understand, based on these comments in the prosecution history, that the claimed vectors in the '179 Patent can encompass vectors with LCR regions that differ in size and in sequence identity from the 3.2 kb LCR fragment disclosed in the Plotkin Declaration, provided that these differences do not bring the LCR fragment to a size that is much greater than 3.2 kb, or materially alter its function. "As apparent from the reference sequence, and as known in the art, ***the three fragments that form the 3.2-kb portion of the LCR are assembled from non-contiguous portions of the LCR***. In this regard, it should be recognized that these fragments can be joined in either 5'-3' or 3'-5' orientation using any of numerous techniques known to those of skill in the art to provide further vector examples. ***Once assembled into a vector, the fragments need not be cleavable nor must***

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<tr><th colspan="2">US 7541179</th><th>SNS23 Vectors</th></tr>
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> ***the entire restriction recognition site be present***. For example, one skilled in the art will readilty [sic] appreciate that the full restriction site might not be present if the fragment is blunt-ended before ligation, yet it may be present if the cut site is filled before ligation." 09/12/2007 Response, at pp.14-15 (italics and emphasis added).

A POSA would also readily understand that sequence for a human gene, particularly a gene such as human β-globin, will change over time as sequencing techniques and technologies improve. Applicants admitted that the "globin genes were among the first ever sequenced at the nucleotide level." 9/12/2007 Response, at p. 7. Applicants themselves admitted in 2007 that "When accessing NG_000007 at present, one obtains version 3, which in relevant part includes an an additional approximately 9kb upstream of the version 1. ***Hence the numbering of the nucleotides is offset between the versions, and can be further slightly offset by polymorphisms and minor variations***." *Id*. (italics and emphasis added). This, combined with the fact that the claim refers to "a" human β-globin LCR rather than "the" human β-globin LCR, would indicate to a POSA that it is the size of the HS-spanning fragments that is most important, and sequence variation in the HS-spanning LCR fragment of the claimed vectors is permitted, provided that it does not substantially alter the vector's properties.

The '433 Publication discloses that each of the SNS23 Vectors is "operably linked" to a nucleotide fragment which consists essentially of three contiguous nucleotide fragments obtainable from a human β-globin locus control region (LCR)". For example, it states:

> "[0246] *SNS23.2.B87.A1, which comprises an expression cassette that comprises a human* $\beta^{A\text{-}T87Q}$ *globin gene, which is operably linked to a β-globin LCR* that comprises a 816 bp HS2 region e.g., one having the nucleotide sequence set forth in SEQ ID NO: 33), a 1301 bp HS3 region e.g., one having the nucleotide sequence set forth in SEQ ID NO: 34), and 754 bp HS4 region (e.g., one having the nucleotide sequence set forth in SEQ ID NO: 35); wherein the β-globin does not comprise a HS1 region;
>
> ...
>
> [0250] FIG. 15 shows five exemplary recombinant vectors *including . . . SNS23.B87.A1, which comprises an expression cassette that comprises a human* $\beta^{A\text{-}T87Q}$ *globin gene, which is operably linked to a β-globin LCR* that comprises a 816 bp HS2 region e.g., one having the nucleotides 45 to 860 of SEQ ID NO: 9), a 1301 bp HS3 region (e.g., one having the nucleotide sequence set

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US 7541179		SNS23 Vectors
		forth in SEQ ID NO: 5), and 754 bp HS4 region (e.g., one having nucleotides 115 to 868 of SEQ ID NO: 6)....” *Id.*, at [0246] and [0250] (italics added). Thus, from the ’433 Publication and their general knowledge, a POSA would understand that: (1) The SNS23.B87.A1 vector has an LCR region that is made up of HS2, HS3 and HS4 fragments that total 2871 bp when combined: 816 bp HS2 fragment +1301 bp HS3 fragment <u>+ 754 bp HS4 fragment</u> Total = 2871 base pairs (2871 bp) As 1000 base pairs equal 1 kilobase (1 kb), 2871 bp equals 2.871 kb, which his falls within the permissible LCR range (greater than 1.0 kb and less than about 3.2 kb) that is defined by the prosecution history this claim; and (2) The SNS23.2.B87.A1 vector also has an LCR region that is made up of HS2, HS3 and HS4 fragments that when combined total 2871 bp: 816 bp HS2 fragment +1301 bp HS3 fragment <u>+ 754 bp HS4 fragment</u> Total = 2871 base pairs (2871 bp), which as shown above is 2.871 kb. This total also falls within the permissible LCR range (greater than 1.0 kb and less than about 3.2 kb) that is defined by the prosecution history of this claim. *See id.*, at [0246] and [0250] and Figures 14 and 15.
1.3	the three fragments being a BstXI and SnaBI HS2-spanning	The SNS23.B87.A1 and SNS23.2.B87.A1 recombinant vectors each meet the claim limitation of “the three fragments being a BstXI and SnaBI HS2-spanning nucleotide fragment of said LCR”.

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<tr><td></td><td>nucleotide fragment of said LCR,</td><td>

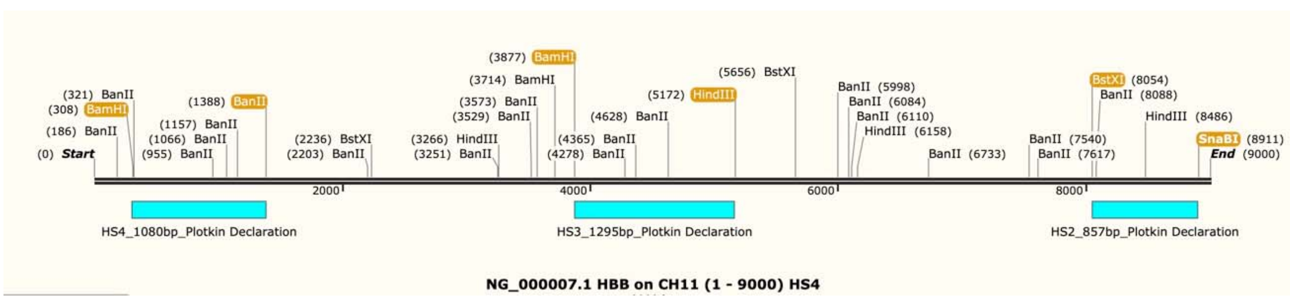
<u>Exemplary Support</u>:

Each of these vectors performs the same function (enhancing β-globin expression beyond levels previously achieved), in the same way (to obtain the same result): through the incorporation of fragments of the HS2, HS3 and HS4 DNase I hypersensitive sites obtained from a human β-globin control region, which fragments are larger than the previously tested minimal HS core elements but smaller than about 3.2 kb when combined.

As the Applicants explained in detail during prosecution of the ’221 Application that matured into the ’179 patent, the claimed vector “comprises a 3.2-kb portion of *a human β-globin locus control region (LCR)* ***consisting essentially of*** *3 restriction fragments*. Each fragment spans a particular DNase I hypersensitive site (HS) and each fragment’s end is identified by particular restriction enzyme recognition sites (listed in 5’ to 3’ order).” 09/12/2007 Response, at p. 6 (italics and emphasis added).

The Applicants submitted the declaration of Mr. Jason Plotkin, a Research Assistant in one of the Inventor’s labs (Dr. Michel Sadelain), which detailed how one could “identify and map the three recited restriction fragments based on the information in the specification, the scientific literature and the reference sequences available as of June 29, 2001,” which is the earliest filing date of the ’221 Application. This declaration used the NG000007.1 human β-globin reference sequence (hereafter, “NG7.1”). *See* Plotkin Declaration, ¶¶ 18 and 29.

A POSA can take this same β-globin reference sequence (NG7.1) and use commonly available vector mapping software to map the HS fragments identified in Mr. Plotkin’s Declaration onto its human β-globin LCR region. *See* Plotkin Declaration, at ¶¶ 38, 44 and 46, and the HS fragment map below:



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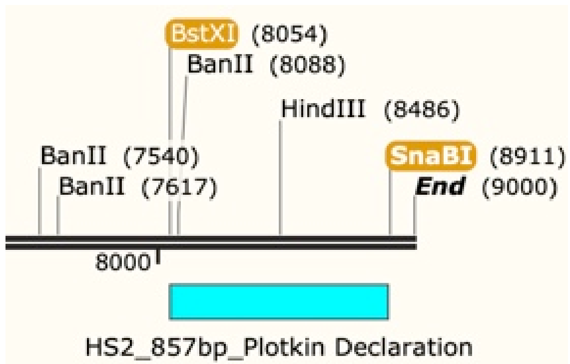
US 7541179		SNS23 Vectors
		The restriction sites recited in claim 1 and identified as bounding the three HS fragments are highlighted in orange (SnaBI, BstXI, HindIII, BamHI, Ban II and BamHI). The size of each originally-identified HS fragment is shown just beneath the blue box that marks its location within the NG7.1 sequence. A close-up view of this HS fragment map, which focuses on the far right region encompassing the HS2 fragment, is provided below:  The HS2 segment, represented by the blue box, is 857 base pairs in length. It is bordered by the SnaBI restriction enzyme recognition site on the right, and the BstXI restriction enzyme recognition site on the left. Based on the disclosures of the '179 Patent, the '433 Publication, and a POSA's general knowledge, a POSA would understand that the HS2 sequence within the SNS23.2.B87.A1 vector is equivalent to a "SnaBI and BstXI HS3-spanning nucleotide fragment of said LCR", (*e.g.*, the HS2 fragment identified in the Plotkin Declaration), because it has the same function and performs this function in the same way to produce the same result – improved transcription of the neighboring β-globin gene within the vector. Likewise, since the SNS23 Vectors share the identical HS2 sequence, the same analysis applies to the SNS23.B87.A1 vector as well.
1.4	a BamHI and HindIII HS3-spanning nucleotide	The SNS23.B87.A1 and SNS23.2.B87.A1 recombinant vectors each meet the claim limitation for "a BamHI and HindIII HS3-spanning nucleotide fragment of said LCR".

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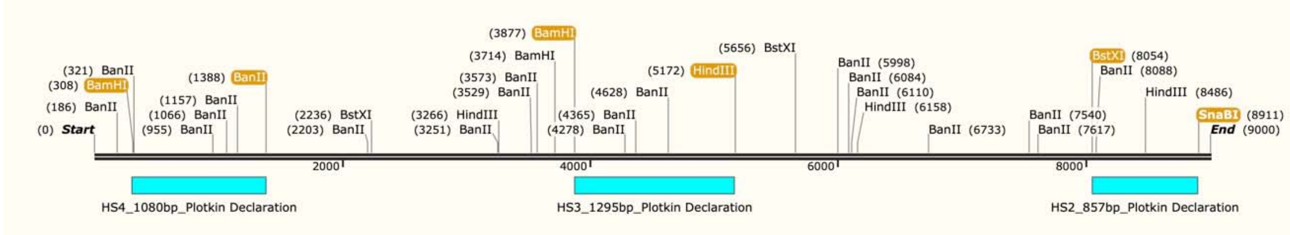
<table>
<tr><th colspan="2">US 7541179</th><th>SNS23 Vectors</th></tr>
<tr><td></td><td>fragment of said LCR</td><td>
<u>Exemplary Support</u>:

Each of these vectors performs the same function (enhancing β-globin expression beyond levels previously achieved), in the same way (to obtain the same result): through the incorporation of fragments of the HS2, HS3 and HS4 DNase I hypersensitive sites obtained from a human β-globin control region, which fragments are larger than the previously tested minimal HS core elements but smaller than about 3.2 kb when combined.

As the Applicants explained in detail during prosecution of the '221 Application that matured into the '179 Patent, the claimed vector "comprises a 3.2-kb portion of *a human β-globin locus control region (LCR)* ***consisting essentially of*** *3 restriction fragments*. Each fragment spans a particular DNase I hypersensitive site (HS) and each fragment's end is identified by particular restriction enzyme recognition sites (listed in 5' to 3' order)." 09/12/2007 Response, at p. 6 (italics and emphasis added).

The Applicants submitted the declaration of Mr. Jason Plotkin, a Research Assistant in one of the Inventor's labs (Dr. Michel Sadelain), which detailed how one could "identify and map the three recited restriction fragments based on the information in the specification, the scientific literature and the reference sequences available as of June 29, 2001," which is the earliest filing date of the '221 Application. This declaration used the NG000007.1 human β-globin reference sequence (hereafter, "NG7.1"). *See* Plotkin Declaration, at ¶¶ 18 and 29.

A POSA can take this same β-globin reference sequence (NG7.1) and use commonly available vector mapping software to map the HS fragments identified in Mr. Plotkin's Declaration onto its human β-globin LCR region. *See* Plotkin Declaration, at ¶¶ 38, 44 and 46, and the HS fragment map below:


NG_000007.1 HBB on CH11 (1 - 9000) HS4
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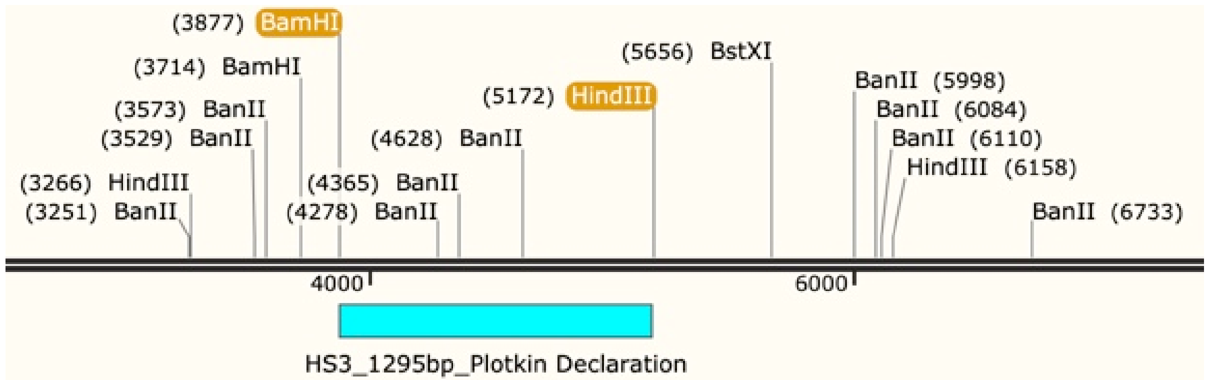
<tr><td></td><td></td><td>The restriction sites recited in claim 1 and identified as bounding the three HS fragments are highlighted in orange (SnaBI, BstXI, HindIII, BamHI, Ban II and BamHI). The size of each originally-identified HS fragment is shown just beneath the blue box that marks its location within the NG7.1 sequence.

A close-up view of this HS fragment map, which focuses on the middle region encompassing the HS3 fragment, is provided below:



The HS3 segment, represented by the blue box, is 1295 base pairs in length. It is bordered by the HindIII restriction enzyme site on the right and the BamHI restriction enzyme site on the left.

Based on the disclosures of the ’179 Patent, the ’433 Publication, and a POSA’s general knowledge, a POSA would understand that the HS3 sequence within the SNS23.2.B87.A1 vector is equivalent to “a BamHI and HindIII HS3-spanning nucleotide fragment of said LCR”, (e.g., the HS3 fragment identified in the Plotkin Declaration), because it has the same function and performs this function in the same way to produce the same result – improved transcription of the neighboring β-globin gene within the vector. Likewise, since the SNS23 Vectors share the identical HS3 sequence, the same analysis applies to the SNS23.B87.A1 vector as well.</td></tr>
<tr><td>1.5</td><td>and a BamHI and BanII HS4-spanning nucleotide</td><td>The SNS23.B87.A1 and SNS23.2.B87.A1 recombinant vectors each meet the claim limitation of “a BamHI and BanII HS4-spanning nucleotide fragment of said LCR”.</td></tr>
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<tr><td></td><td>fragment of said LCR,</td><td>

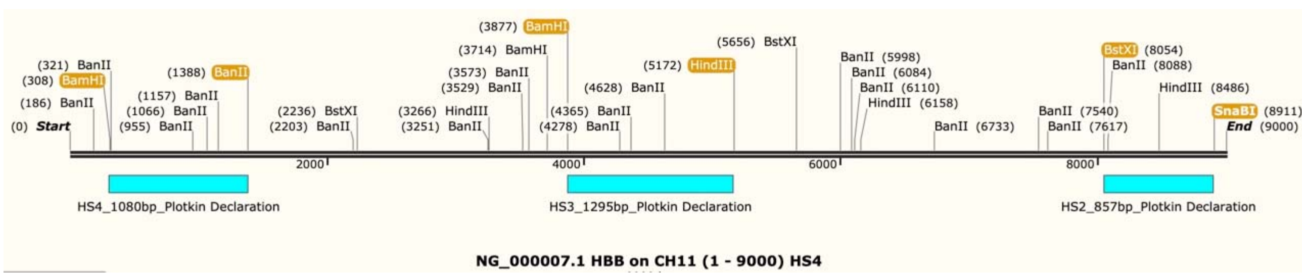
<u>Exemplary Support</u>:

Each of these vectors performs the same function (enhancing β-globin expression beyond levels previously achieved), in the same way (to obtain the same result): through the incorporation of fragments of the HS2, HS3 and HS4 DNase I hypersensitive sites obtained from a human β-globin control region, which fragments are larger than the previously tested minimal HS core elements but smaller than about 3.2 kb when combined.

As the Applicants explained in detail during prosecution of the ’221 Application that matured into the ’179 Patent, the claimed vector “comprises a 3.2-kb portion of *a human β-globin locus control region (LCR)* ***consisting essentially of*** *3 restriction fragments*. Each fragment spans a particular DNase I hypersensitive site (HS) and each fragment’s end is identified by particular restriction enzyme recognition sites (listed in 5’ to 3’ order).” 09/12/2007 Response, at p. 6 (italics added) (emphasis added).

The Applicants submitted the declaration of Mr. Jason Plotkin, a Research Assistant in one of the Inventor’s labs (Dr. Michel Sadelain), which detailed how one could “identify and map the three recited restriction fragments based on the information in the specification, the scientific literature and the reference sequences available as of June 29, 2001,” which is the earliest filing date of the ’221 Application. This declaration used the NG000007.1 human β-globin reference sequence (hereafter, “NG7.1”). *See* Plotkin Declaration, at ¶¶ 18 and 29.

A POSA can take this same β-globin reference sequence (NG7.1) and use commonly available vector mapping software to map the HS fragments identified in Mr. Plotkin’s Declaration onto its human β-globin LCR region. *See* Plotkin Declaration, at ¶¶ 38, 44 and 46, and the HS fragment map below:



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</td></tr>
</table>

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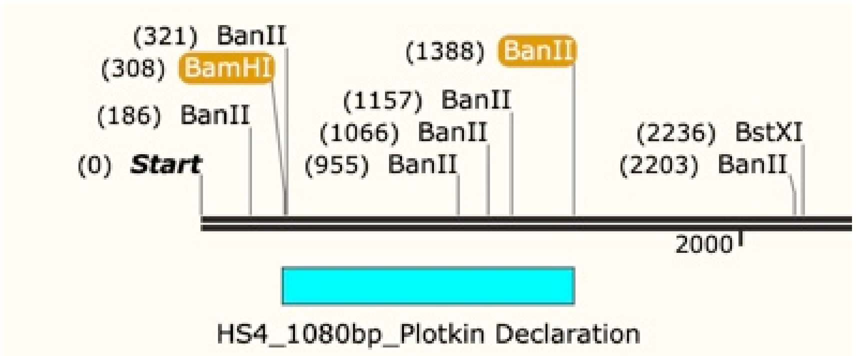
		A close-up view of this HS fragment map, which focuses on the far left region encompassing the HS4 fragment, is provided below:  The HS4 segment, represented by the blue box, is 1080 base pairs in length. It is bordered by the BanII restriction enzyme site on the right and the BamHI restriction enzyme site on the left. Based on the disclosures of the ’179 Patent, the ’433 Publication, and a POSA’s general knowledge, a POSA would understand that the HS4 sequence within the SNS23.2.B87.A1 vector is equivalent to “a BamHI and BanII HS4-spanning nucleotide fragment of said LCR”, (*e.g.*, the HS4 fragment identified in the Plotkin Declaration), because it has the same function and performs this function in the same way to produce the same result – improved transcription of the neighboring β-globin gene within the vector. Likewise, since the SNS23 Vectors share the identical HS4 sequence, the same analysis applies to the SNS23.B87.A1 vector as well.
1.6	said vector providing expression of the globin in a mammal in vivo.	The SNS23.B87.A1 and SNS23.2.B87.A1 recombinant vectors each meet the claim limitation where “said vector providing expression of the globin in a mammal in vivo.” Exemplary support: *See* claim 1.1 *supra*. *See also*, ’433 Publication, [0355] and Table 2, plus Figure 19 and [0062], which are all shown below. The information specific to vector SNS23.2.B87.A1 has been italicized or highlighted in yellow.

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		"*Example 7: Evaluation of Globin Production at Different Time Points* [0355] *Additional experiments were conducted to measure the globin production at different time points in thalassemic mice transfected with vectors disclosed herein*. The methods used in Example 6 were applied in this Example. Average total Hb levels and average gains in Hb levels (ΔHb) in peripheral blood were measured in thalassemic mice transfected with vectors for 6-week, 5-month, or 9-month [sic]. ΔHb was normalized to vector copy (VCN), and ΔHb=Hb level-7.5 (baseline level in thalassemic mice, as all the time points consistently show an HGB value of 7.5 g/dL in the Thalassemic mice used as controls, [so] this value was used). *As shown in FIG. 19 and Table 2, globin production from all vectors, including SNS23.2.B87.A1, were stable over time. . . .*"

TABLE 2

Representative data for three different time points after transplant

Vectors	VCN	HGB (g/dL)	Δ(HGB-7.5)	ΔHGB/ copy
TNS9.B87.A1 (6 weeks)	0.5	10.6	3.8	8.6
TNS9.B87.A1 (5 months)	0.5	10.7	3.2	5.1
TNS9.B87.A1 (9 months)	0.4	9.8	2.3	6.4
SNS23.2.B87.A1 (6 weeks)	1.1	13.2	5.7	6.0
SNS23.2.B87.A1 (5 months)	1.1	11.3	3.8	4.3
SNS23.2.B87.A1 (9 months)	0.5	11.6	4.1	8.3
SNS26.B87.A1 (6 weeks)	2.0	11.2	3.7	2.0
SNS26.B87.A1 (5 months)	3.0	11.1	3.6	1.5
SNS26.B87.A1 (9 months)	3.1	11.1	3.6	1.4
SNS27.2.B87.A1 (6 weeks)	0.8	11.8	4.3	5.3
SNS27.2.B87.A1 (5 months)	1.5	12.1	4.6	3.5
SNS27.2.B87.A1 (9 months)	1.1	10.4	2.7	2.4
TH3/+ MOCK (6 weeks)	0	7.5	0	0
TH3/+ MOCK (5 months)	0	7.5	0	0
TH3/+ MOCK (9 months)	0	7.5	0	0

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	Average of Vector copy number (VCN) in long-term hematopoietic chimeras. Average Hb level [g/dL] in peripheral blood (PB) of chimeric mice. ΔHb level was obtained by subtracting Th3/+ hemoglobin value (value=7.5 g/dL) from total Hb level for each animal tested. ΔHGb/copy=Correlation between delta (Δ)Hb and vector copy number. All the ΔHGb calculations are made using TH3/+ MOCK (7.5 g/dL) as basal HGB, value that is consistent in all the time points. ’433 Publ., at [0355] (Table 2 and legend) (italics and emphasis added). *See also* Figure 19 of the ’433 Publication, as reproduced below.

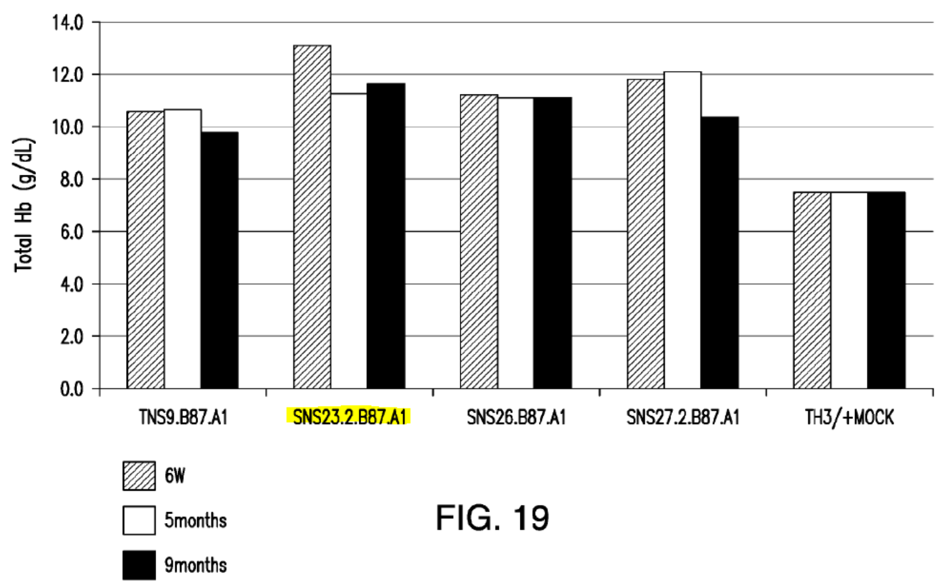

FIG. 19

“FIG. 19 depicts the average of total Hemoglobin (Hb) in thalassemic mouse peripheral blood at time points of 6-week, 5-month, and 9-month [sic].” ’433 Publ., at [0062].

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US 7541179		SNS23 Vectors
23.0	A recombinant vector comprising	The SNS23.B87.A1 and SNS23.2.B87.A1 vectors are each "a recombinant vector." Exemplary support: *See* claim 1.0 *supra*.
23.1	a nucleic acid encoding a functional globin	The SNS23.B87.A1 and SNS23.2.B87.A1 recombinant vectors each have "a nucleic acid encoding a functional globin". Exemplary support: *See* claim 1.1 *supra*.
23.2	operably linked to a 3.2-kb nucleotide fragment which consists essentially of three nucleotide fragments obtainable from a human β-globin LCR,	The SNS23.B87.A1 and SNS23.2.B87.A1 recombinant vectors each meet the limitation of "operably linked to a 3.2-kb nucleotide fragment which consists essentially of three nucleotide fragments obtainable from a human β-globin LCR". Exemplary support: *See* claim 1.2 *supra*.
23.3	the three fragments being a BstXI and SnaBI, HS2-spanning nucleotide fragment of said LCR,	The SNS23.B87.A1 and SNS23.2.B87.A1 recombinant vectors each meet the limitation of "the three fragments being a BstXI and SnaBI, HS2-spanning nucleotide fragment of said LCR". Exemplary support: *See* claim 1.3 *supra*.

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23.4	a BamHI and HindIII, HS3-spanning nucleotide fragment of said LCR,	The SNS23.B87.A1 and SNS23.2.B87.A1 recombinant vectors each meet the limitation of "a BamHI and HindIII, HS3-spanning nucleotide fragment of said LCR". Exemplary support: *See* claim 1.4 *supra*.
23.5	and a BamHI and BanII, HS4-spanning nucleotide fragment of said LCR,	The SNS23.B87.A1 and SNS23.2.B87.A1 recombinant vectors each meet the limitation of "and a BamHI and BanII, HS4-spanning nucleotide fragment of said LCR". Exemplary support: *See* claim 1.5 *supra*.
23.6	wherein the HS3-spanning nucleotide fragment and the HS4-spanning nucleotide fragment are adjacent to each other and the vector further comprises 2 GATA-binding sites at the junction between the HS3-spanning and HS4-spanning	The SNS23.B87.A1 and SNS23.2.B87.A1 recombinant vectors each meet the claim limitation "wherein the HS3-spanning nucleotide fragment and the HS4-spanning nucleotide fragment are adjacent to each other". Exemplary Support: During prosecution of the '221 patent application that matured into the '179 Patent, the Applicants defined "adjacent" as follows: "Claim 1 as currently amended recites that the three HS fragments are contiguous. According to the Random House Dictionary, contiguous is defined as: 1. touching; in contact, or 2. *in close proximity without actually touching; near.* The Random House Dictionary of the English Language (Stein, Ed.) Random House, New York, 1973, p. 316. For the first meaning, the definition is synonymous with bordering, adjoining and abutting; *for the second, the definition is synonymous with adjacent*. That the three fragments are contiguous is clear from Figs. 1 and 2. Amending the claim in this manner makes explicit a relationship that was already implicit in the subject matter as previously claimed. As discussed in the text, here and in previous

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	nucleotide fragments,	responses, the size sum of the three HS fragments rounds to 3.2 kb, meaning that the fragments are at least adjacent and could easily be adjoined." 06/03/2008 Amendment After Final Office Action in US Patent Application No. 10/188,221, at p. 8 (italics added). Based on pairwise analyses of the SNS23 Vector sequences as disclosed in the '433 Publication, a POSA would readily determine that this recited feature of two GATA-binding sites is found in the SNS23 Vectors**, but it sits between the HS2 and HS3 fragments instead of between the HS3 and HS4 fragments, as recited in this claim limitation**. Despite this, a POSA would readily understand that this feature of the SNS23 Vectors performs the same function (of providing a binding site for GATA-transcription factor/s) in the same way (through the use of two specific GATA-binding sites) to achieve the same result of improved β-globin transcription. Binding of the GATA transcription factor/s at the junction between the HS2 and HS3 fragments still achieves the same result as when the GATA transcription factor/s bind between HS3 and HS4: the increased transcription of the β-globin gene located in the vector. The details to support this analysis are provided in the sequence alignments on the following pages. Based on a pairwise alignment of the SNS23.2.B87.A1 vector sequence against the HS2 and HS3 nucleotide sequences provided in the '433 Publication (as shown below), a POSA would readily determine that there are two GATA binding sites in the region between the end of the HS2 fragment and the start of the HS3 fragment:

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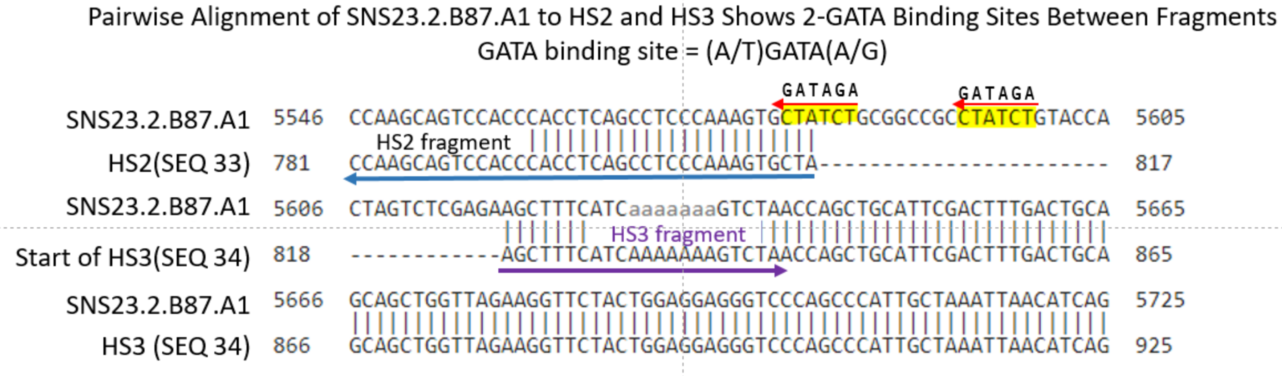
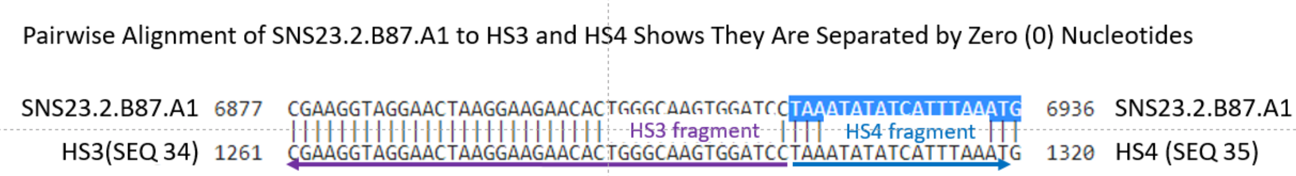


In this sequence alignment above, the tail end of the HS2 fragment is underlined with a blue left-facing arrow, and the start of the HS3 fragment is underlined with a purple right-facing arrow. The two GATA binding sites are indicated with red left-facing arrows, and they are also highlighted in yellow. A POSA would readily understand that the DNA complementary sequence for CTATCT (highlighted in yellow) would be GATAGA, which is a GATA transcription factor binding site.

In addition, a POSA would also readily understand (based on a simple pairwise alignment of the SNS23.2.B87.A1 vector sequence against the HS3 and HS4 nucleotide sequences provided in the '433 Publication), that there are no nucleotides between the end of the HS3 fragment and the start of the HS4 fragment. This is illustrated in the partial sequence alignment below.



In this alignment, the tail end of the HS3 fragment end is in underlined in purple with a left-facing arrow, and the HS4 start site is underlined in blue with a right-facing arrow. The SNS23.2.B87.A1 vector sequence (SEQ ID NO: 39) is shown on the top line, and the HS3/HS4 sequences (SEQ ID NOS: 34 and 35, respectively) are from the '433 Publication.

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US 7541179		SNS23 Vectors
		A POSA looking at this alignment would readily comprehend that there are no nucleotides separating the HS3 from the HS4 regions in the SNS23.2.B87.A1 vector. As the HS3 and HS4 sequences used for the SNS23.2.B87.A1 vector are the same as those for the SNS23.B87.A1 vector in these regions, the same analysis applies.
23.7	said vector providing expression of the globin in a mammal in vivo.	The SNS23.B87.A1 and SNS23.2.B87.A1 recombinant vectors each meet the claim limitation of "said vector providing expression of the globin in a mammal in vivo". Exemplary Support: *See* claim 1.6 *supra*.

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